

Application of otolith chemistry to studies of fish movement and migration

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Abstract

In the past decade, research has confirmed that otoliths contain a chemical record that can be interpreted in relation to the past life of individual fish. The extent to which this chemical record can be considered an undistorted log of past environments and, concomitantly, movements experienced by a fish, is still open to debate. Furthermore, the complexity of this debate has increased dramatically in recent years. Otolith chemistry has progressed from measurement of major trace elements in whole otoliths by solution-based atomic absorption spectrophotometry, to the quantification of individual isotopes at ultra-trace levels with quadrupole mass spectrometers linked to laser ablation systems. This advance represents the jump from identifying changes in the gross chemical composition of individual otoliths, to the determination of the age at which an individual fish moved into a particular estuary. The instrumental escalation associated with otolith chemistry studies is the result of three key factors: 1) development and increased availability of new analytical instrumentation; 2) a greater appreciation, by fish biologists, of aquatic chemistry and its possible influence on the composition of fish; and 3) increased collaboration with those involved in geochemistry and related disciplines. Although analysis of an individual otolith may have reached new heights in terms of cost and complexity, the detailed information that can now be extracted is

unlikely to be attainable by any other method. Furthermore, these data are realised without manipulation of the fish prior to its death, ensuring a virtually unlimited supply of samples.

Background

Recent research has confirmed that otoliths contain a chemical record that can be interpreted in relation to the past life of an individual fish. Patterns of fish growth, movement and stock separation have been revealed through analysis of the structure and chemistry of otoliths. This record is evident due to the incorporation of particular elements and isotopes into the otolith from the surrounding water in concentrations that reflect the physical and chemical aspects of the ambient environment and the physiology of the fish (Kalish 1990; Fowler *et al.* 1995; Secor *et al.* 1995). Two assumptions are commonly made in studies of otolith chemistry:

- otoliths grow throughout the life of the fish; and,
- otolith material is not resorbed or altered after deposition.

These assumptions are well-supported by a range of otolith studies, and the notion that otoliths contain a permanent record of a fish's environment, tempered in varying degrees by physiology, is established in the otolith literature.

Otoliths, the crystalline 'ear-stones' found in the bony auditory capsules at the back of the cranium, are part of the 'labyrinth', the auditory-equilibrium organ of teleost fishes (Platt and Popper 1981). There are three pairs of otoliths in the inner ear: the sagitta, lapillus and asteriscus. In most fishes, the sagitta is the largest of the three otoliths and is used most often in studies of otolith chemistry.

The major components of otoliths are calcium carbonate, as aragonite, and several otolith proteins including a protein called otolin (Degens *et al.* 1969). While the major component of otoliths is calcium carbonate (approximately 99%), the organic component (< 1%) plays an important role in the deposition of CaCO₃ and incorporation of other elements from the endolymph into the otolith structure.

Little research has been concentrated on the process of mineralisation in otoliths. What is known, however, is that despite being only a small proportion of the otolith, the organic components can affect the variability of metal ions: the organic material is largely anionic protein and hence has a high affinity for cations. Dove *et al.* (1996) found that the ratio of protein to inorganic material varies throughout the otolith and that there are chronological differences in trace elements. Although there are several studies of otolith proteins, the relative affinities that ions have for the organic and inorganic constituents of otoliths have not been determined.

The extent to which the chemical record can be considered an undistorted log of past environments and, concomitantly, movements experienced by a fish, is still open to debate. If otolith composition reflects the water mass in which the fish lives, then the elemental 'fingerprint' could provide a history of fish movements (Kalish 1989). However, many things affect the elemental composition of otoliths including environmental factors, such as temperature and salinity, as well as the physiology of the fish, further influenced by factors such as ontogeny and stress.

The incorporation of elements into the otolith structure

may not occur in a simple way (Kalish 1989; Fowler *et al.* 1995) and the various factors that affect deposition may confound interpretation of results of otolith microchemistry studies. Otoliths grow through the regular deposition of both mineral-rich and organic-rich zones (Campana and Neilson 1985; Mugiya 1987). Nevertheless, there can be large variations in elemental concentrations around the margins of otoliths (Kalish 1989), which indicates that a simple 'onion' model with concentric shells of mineral-rich and organic-rich material may not be adequate to describe otolith growth. This has particular implications for chronological studies that measure otolith chemistry, assumed to be deposited at particular times during the life of a fish (Gunn *et al.* 1992; Dove *et al.* 1996; Kalish *et al.* 1996b).

To fully understand the significance of surrounding water masses in otolith composition and hence to be able to reconstruct the movement and migration patterns of fish from the otolith elemental concentrations, the effects of multiple factors should be considered. In one such study of the anadromous striped bass *Morone saxatilis*, Secor *et al.* (1995) conducted aquarium experiments in which temperature and salinity were manipulated. By measuring elemental concentrations along otolith sections through the transverse plane they were able to determine the extent to which temperature and growth rate affected strontium deposition and quantified the relationship between salinity in the surrounding water and otolith Sr/Ca ratios.

Techniques

Otolith chemistry has progressed from measurement of major trace elements in whole otoliths by solution-based atomic absorption spectrophotometry, to the quantification of individual isotopes at ultra-trace levels with quadrupole mass spectrometers linked to laser ablation systems. This advance has facilitated more complex analyses: from identifying changes in the gross chemical composition of otoliths, to the determination of the age at which an individual fish moved into a particular estuary.

Table 1. Commonly used methods in otolith chemistry research.

Instrument/method	Otolith constituents measured	Method
Stable isotope mass spectrometry (SIMS)	carbon and oxygen isotope ratios	whole otolith
Atomic absorption spectrophotometry (AAS)	micro elements	whole otolith
Energy-dispersive electron microprobe (ED-EM)	macro elements	point analysis of sectioned and polished otoliths
Wave dispersive electron microprobe (WD-EM)	micro elements	point analysis of sectioned and polished otoliths
Proton-induced X-ray emission (PIXE)	trace elements	point analysis of sectioned and polished otoliths
Solution-based inductively coupled plasma-atomic emission spectroscopy (ICP-AES)	trace elements	whole or isolated portion of otolith
Isotope dilution inductively coupled plasma-mass spectrometry (ID-ICP-MS)	trace elements/isotopes	whole otolith
Solution-based inductively coupled plasma-mass spectrometry (ICP-MS)	trace elements/isotopes	whole or isolated portion of otolith
Laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS)	trace elements/isotopes	point analysis of sectioned and polished otoliths
Accelerator mass spectrometry (AMS)	ultra-trace isotopes	whole or isolated portion of otolith

The instrumental escalation associated with otolith chemistry studies is the result of three key factors:

- development and increased availability of new analytical instrumentation;
- a greater appreciation, by fish biologists, of aquatic chemistry and its possible influence on the composition of fish otoliths; and,
- increased collaboration with those involved in geochemistry and related disciplines.

The cost of otolith chemistry research can be high and techniques may produce varied results so a thorough review of methodological considerations should be carried out before choosing the most appropriate method for the required analysis. Which instrument and method are chosen for otolith chemistry analyses depends on the type and concentration of elements being studied (Table 1). In general, the concentration of elements in otoliths can be described as 'macro', >10% by weight, such as Ca, C and O; 'micro', 100-5 000 ppm, including Na, Sr, K, S and Cl; and 'trace',

less than 50-100 ppm, including Zn, Br, Se, Ni and Pb. In addition, otolith research on movement and migration has included analyses of stable isotopes of carbon and oxygen, which are potential indicators of environmental temperature (Kalish 1991; Radtke *et al.* 1996, Edmonds and Fletcher 1997; Edmonds *et al.* 1999; Thorrold *et al.* 1997).

A recent study (Campana *et al.* 1997) provided an inter-laboratory calibration of trace element analyses based on the more popular techniques used in otolith chemistry research. They compared the accuracy, precision and sensitivity of the energy-dispersive electron microprobe (ED-EM), wave dispersive electron microprobe (WD-EM), proton-induced X-ray emission (PIXE) and laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Independent researchers at eight laboratories conducted assays for ten elements of 'blind-labelled' real and artificial otolith samples.

No individual instrument was found to be the most effective for measurement of all elements. However, the authors did reach several conclusions:

- electron microprobe measured 'abundant' elements such as sodium and potassium most accurately;
- trace elements required PIXE or LA-ICPMS; and,
- strontium could be measured accurately and precisely by WD-EM, PIXE and LA-ICPMS.

The authors also make a cautionary note that comparisons between published studies should only be made the *proviso* that adequate calibrations were used, as significant differences were found between the results from different laboratories.

Contamination/effects of handling

The introduction of contaminants into chemical analyses has always been of concern, but the increasing sensitivity of analytical techniques makes avoiding contamination even more pertinent. Recent research

(Milton and Chenery 1998; Proctor and Thresher 1998) has been focussed on possible changes in otolith chemistry due to procedures used to collect and prepare otoliths for chemical analysis. These include:

- storage and preservation of fish before extracting otoliths;
- delay in extracting otoliths;
- preservation of otoliths;
- cleaning; and,
- preparation for analysis.

By exposing otolith pairs to different treatments before WD electron probe analysis, Proctor and Thresher (1998) showed that the six elements tested were sensitive to the treatments; calcium and strontium were least affected by collection and preparation procedures whereas; sodium, potassium, sulphur and chlorine showed significant variation. Post-capture storage has also been found to affect results of LA-ICPMS analyses. Milton and Chenery (1998) compared concentrations of elements at the primordium and otolith edge and found that, for four of the seven elements tested, method of storage had measurable effects on the concentrations.

Applications of techniques relevant to movement and migration

Applications of recent developments in otolith chemistry analysis have been effective in studies of anadromous and non-anadromous fish (Kalish 1990 using WD-EM; Radtke *et al.* 1996 using WD-EM; Babuluk *et al.* 1997 using PIXE), the results of which have important implications for the management of fish populations in river and lake systems.

In studies of species that display facultative anadromy, such as Arctic charr *Salvelinus alpinus* and rainbow trout *Oncorhynchus mykiss*, otolith composition can differentiate groups of anadromous and non-anadromous fish. Distinct patterns of strontium

distribution associated with two life histories are revealed when comparing otolith strontium levels measured along transects between the primordium (earliest-formed) and outside edge (latest-formed). This knowledge can be applied to investigations of groups whose life histories are 'unknown', providing evidence that the fish are either anadromous or non-anadromous and even at what point in their lives they entered particular estuaries.

The otolith chemistry of the material deposited during the earliest part of life can reveal information about fish population structure: the natal area and coastal migrations. As trace elements are incorporated into otoliths as the fish grows, different otolith elemental composition can reflect environments or water masses that have different physical and chemical characteristics.

Site-specific otolith chemistry has been detected in some species. It has been shown to exist in yellowfin tuna *Thunnus albacares*, and can be detected using WD-EM and PIXE (Gunn and Ward 1994). Young-of-the-year were sampled at spawning sites throughout the western Pacific and analysed for otolith chemical variation. Seven of the 15 elements found in the otoliths exhibited significant variation among sites. Interestingly, the samples from the two semi-tropical sites, Coral Sea and Hawaii, were most similar. This suggests that the elemental variation measured in otoliths from fish collected at different sites may be associated with factors other than ambient water chemistry, such as growth rate (Kalish 1989; Sadovy and Severin 1992; Fowler *et al.* 1995).

Understanding the origins of individuals within a fishery is a question asked commonly by fishery managers. The 'elemental fingerprint' has been used to identify the origins of sub-groups within fishery stocks and to determine the extent of mixing of different populations (Edmonds *et al.* 1989, 1991; Campana *et al.* 1994). Campana *et al.* (1995) used ID-ICPMS to identify Atlantic cod *Gadus morhua* that originated from the Gulf of St Lawrence within the fishery off the coast of eastern Canada. Elemental fingerprints, however, are not always effective for the

identification of unique stocks (Thresher *et al.* 1994; Kalish *et al.* 1996a). In most cases the basis for the measured differences in otolith composition among putative stocks is not understood despite its usefulness in stock discrimination, and further research is essential to provide a stronger foundation for these investigations.

Variation in otolith composition can be useful for understanding the migration of fish from their spawning area. Using LA-ICPMS analysis, Milton *et al.* (1997) investigated the otolith microchemistry of the tropical shad, terubok *Tenulosa toli*, thought to originate from only two spawning estuaries. They compared the composition of otolith nuclei of fish collected at eight coastal sites and the two known spawning areas. Evidence of one or more additional spawning areas was revealed the otolith chemistry of fish from two of the eight sites was significantly different from the otolith chemistry of the fish collected in the estuaries.

Conclusions

Although analysis of an individual otolith may have reached new heights in terms of cost and complexity, the detailed information that can now be extracted is unlikely to be attainable by any other method. The development of ICPMS techniques during the last decade has been a major advance in detection of trace elements in otolith chemistry studies. Over the next few years, modifications to the instrument should bring improvements in sensitivity and a reduction in interferences for many elements. A further development to magnetic-sector and multiple-collector ICPMS will allow precise isotopic ratios to be determined for stable isotopes and elements such as strontium (Halliday *et al.* 1998).

The recent investigations of possible changes in otolith chemistry due to handling and preparation should lead to an awareness of possible post-mortem artefacts and their effect on levels of trace metal concentrations. These results challenge the assumption that otoliths are inert and permanently retain a true record of the

otolith chemistry deposited during the life of a fish. A further, related challenge is to improve the understanding of otolith structure, the differential deposition of trace elements into the protein matrix and aragonitic crystalline structure, and the labile nature of particular elements.

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